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Pituitary beta-endorphin levels and naloxone effects on feeding in several experimental obesity syndromes

by

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Abstract

Recent research has suggested that endorphin systems may participate in the physiological regulation of feeding behavior in both normal and genetically obese animals. The purpose of this research was to both examine the effects of the opiate antagonist naloxone on feeding behavior and measure pituitary beta-endorphin-like immunoreactivity for each of several animal models of obesity.

Adult female rats received ventromedial hypothalamic lesions (VMH), dorsolateral tegmental lesions (DLT), parasagittal hypothalamic knife cuts (KC), intraventricular injections of 5,7-dihydroxytryptamine (5,7-DHT), or control surgery (NWRA), or were ovariectomized (OVX). All animals were maintained at normal body weight during testing except for an additional group of neurologically intact rats that was maintained at 75% of normal body weight (DEP). The effect of 0.5, 1.8, 6.8, and 25.0 mg/kg naloxone on the consumption of laboratory pellets was measured during four-hour daily access periods. Doses of 1.8, 6.8, and 25.0 mg/kg caused successive significant decrements in food intake. Groups KC, 5,7-DHT, DLT, and VMH ate significantly more than group NWRA. Groups OVX and DEP did not eat more than NWRA. The Group x Dose interaction was not significant. Thus, while both naloxone dose and surgery had significant effects on food intake, no surgical group was more or less

sensitive to the effects of naloxone on food intake than any other.

After completion of the naloxone tests half of each surgical group continued maintenance on pellets at normal body weights. The other half of each surgical group was allowed to feed ad libitum on a wet mash diet (70% water). Additional rats from the same original pool served as another control group. These rats had never received naloxone and had always had free access to laboratory pellets. Half of these rats continued on pellets ad libitum and half began free access to the wet mash. After five weeks animals were sacrificed and pituitaries assayed for beta-endorphin-like immunoreactivity. Pellet-fed rats had slightly lower (11%) concentrations of beta-endorphin-like immunoreactivity than wet mash-fed rats. This effect appeared due to diet itself rather than differences in body weight or feeding regimen. Rats with hypothalamic knife cuts had significantly lower pituitary beta-endorphin-like immunoreactivity than all other groups.

These results indicate: (1) that at lean body weights, feeding in the hyperphagia/obesity syndromes tested is not more or less sensitive to naloxone than in normal rats; (2) that elevated concentrations of beta-endorphin-like immunoreactivity are not related to hyperphagia in the syndromes examined; and (3) that obesity itself may have

little if any effect on pituitary beta-endorphin-like immunoreactivity.

Introduction

The recent discovery of two groups of peptides with opioid properties, the enkephalins and endorphins, has stimulated a flurry of varied research activities. In the four years since their discovery the possible roles of these substances in pain modulation (Cox, Goldstein, & Li, 1976; Graf, Szekely, Ronai, Dunai-Kovacs, & Bajusz, 1976), mental illness (Vereby, Volavka, & Clouet, 1978; Watson, Akil, Berger, & Barchas, 1979), sexual behavior (Gessa, Paglietti & Quarantotti, 1979), open field activity (Veith, Sandman, Walker, Coy, & Kastin, 1978), stress responses (Guillemin, Vargo, Rossier, Minick, Ling, Rivier, Vale, & Bloom, 1977), the regulation of gut activities (Hughes, Smith, Kosterlitz, Fothergill, Morgan, & Morris, 1975; Jacques, 1977; Konturek, Pawlik, Walus, Coy, & Schally, 1978; Konturek, Tasler, Cieszkowski, Jaworek, Coy, & Schally, 1978), and control of food intake (Grandison & Guidotti, 1977; Kenny, McKay, Woods, & Williams, 1978; Margules, Moisset, Lewis, Shibuya, & Pert, 1978) have been examined. Investigations in the latter category have yielded intriguing results, and have suggested a role particularly for beta-endorphin (B-END) in both normal and abnormal feeding behaviors.

B-END is contained in high concentrations in both the hypothalamus and pituitary (Bloom, Rossier, Battenberg, Bayon, French, Henriksen, Siggins, Segal, Browne, Ling, &

Guillemin, 1978). There is suggestive evidence that B-END in both locations may play a role in feeding behavior. Kenny et al. (1978) reported that injection of 200 ng B-END into the lateral ventricle of the rat increased consumption of sweetened milk. This effect was not obtained with other peptides (cholecystokinin, substance P, neurotensin). Peripheral injection of the same dose of B-END was without effect. Grandison and Guidotti (1977) demonstrated that intrahypothalamic injection of 1.46 nm of B-END caused increased intake of laboratory chow. If the effects of B-END on feeding behavior are specific to opiate receptor activation, then drugs which prevent the binding of B-END to its receptors should block the effects of exogenous B-END. Grandison and Guidotti (1975) reported that intrahypothalamic injection of naloxone, a drug known to compete "more successfully" than B-END for the same receptors, blocked the feeding behavior induced by B-END.

Naloxone also suppresses the feeding behavior of intact animals. Holtzman (1974, 1975) reported that naloxone suppressed intake of laboratory pellets during feeding after 48-hour food deprivation, and also decreased the intake of sweetened Enfamil in nondeprived rats. Diaz, Paul, Frenk, and Bailey (1978) found that naloxone inhibited intake of evaporated milk in rats whose total caloric and fluid intake consisted of one-hour access to the milk. Margules et al.

(1978) found that naloxone suppressed food intake of normal rats and mice on a two or four hour restricted access schedule. These data suggest that B-END, or some other endogenous substance which binds to the same receptors, has a role in normal feeding.

Margules et al. (1978) also investigated the effects of naloxone on the food intake of genetically obese mice (ob/ob) and rats (fa/fa). Food intake of genetically obese animals was decreased to a greater degree than was that of lean littermates, and significant suppression of feeding was obtained with lower doses of naloxone in genetically obese animals than in lean littermates. The significance of the greater effect of naloxone in genetically obese animals was magnified by data showing that the genetically obese animals had concentrations of B-END in their pituitaries 75 to 80% higher than those of lean littermates. Hypothalamic B-END content of the obese rodents did not differ from lean littermates.

The findings of Margules et al. (1978) clearly raise the possibility that actions of excessive pituitary B-END may be related to the exaggerated feeding of these genetically obese animals. Recent work by Ipp, Dobbs, and Unger (1978) suggests a mechanism by which excessive B-END might cause greater than normal food intake. Ipp et al. (1978) reported that B-END causes release of insulin in vitro from isolated

pancreatic islets. This suggests a primary hyperinsulinemia as a mechanism in genetic obesity. Moreover, Beloff-Chain and colleagues (Beloff-Chain, Edwardson, & Hawthorn, 1975; Beloff-Chain & Hawthorn, 1976; Beloff-Chain, Hawthorn, & Green, 1975) have shown that some material in pituitary perifusate causes in vitro release of insulin from pancreatic islets. Perifusates of pituitaries from genetically obese mice were more effective insulin secretagogues than perifusates from pituitaries of lean littermates. In summary, the above data suggest that B-END mechanisms may participate in the regulation of feeding behavior in both normal and genetically obese animals. One question raised by these data is the extent to which abnormalities in B-END systems may be associated with experimentally-induced obesities caused by central or peripheral interventions. There appear to be few data applicable to this question.

Obesity can be induced by a number of interventions, including lesions of the ventromedial hypothalamus (Hetherington & Ranson, 1940), knife cuts between the medial and lateral hypothalamus (Sclafani, Springer, & Kluge, 1976), lesions of the dorsolateral tegmentum (Oltmans, Lorden, & Margules, 1977; Peters, Gunion, & Wellman, 1979), intraventricular injection of 5,7-dihydroxytryptamine (5,7-DHT; Saller & Stricker, 1976), and ovariectomy (Zucker, 1969). No reports examining the effects of B-END antagonists

on feeding in these syndromes appear to exist, nor do data concerning pituitary B-END levels in these syndromes. Only two reports appear to bear on this question at all; both were investigations of the role of the pituitary in obesity development. York and Bray (1972) demonstrated that lesions of the ventromedial hypothalamus (VMH) induce overeating and obesity in both hypophysectomized and intact animals. Ahlskog, Hoebel, and Breisch (1974) examined pituitary involvement in obesity due to lesions of the dorsolateral tegmentum (DLT) and found that hypophysectomy blocked the development of this obesity.

The reports of York and Bray (1972) and Ahlskog et al. (1974) suggest that VMH and DLT lesion syndromes may differ with respect to involvement of B-END, if abnormally high concentrations of pituitary B-END are indeed of importance in obesity development. VMH-lesioned rats might be expected to have normal levels of pituitary B-END, since the pituitary is not necessary for the development of VMH obesity. DLT-lesioned rats, however, might be expected to have elevated levels of pituitary B-END, since the pituitary is required for the development of this obesity. As a corollary, it might also be expected that the feeding of VMH-lesioned rats would be no more sensitive to the effects of naloxone than the feeding of normal rats, while the

feeding of DLT-lesioned rats would be significantly more suppressed.

Methods

General design

The experiment described here was designed (1) to test the effect of naloxone on the feeding behavior of several experimental models of obesity, and (2) to see if any differences in pituitary B-END concentrations exist among these models. To avoid any confounding effects of obesity, animals were maintained at normal body weights throughout naloxone testing. After naloxone testing pituitaries were assayed for B-END content. The general course of the experiment was:

1. surgery
2. recovery
3. test for naloxone effects on feeding
4. maintenance until sacrifice.

Groups. Five overeating/obesity syndromes were investigated. These were the syndromes due to:

1. lesions of the ventromedial hypothalamus (VMH)
2. lesions of the dorsolateral tegmentum (DLT)
3. wire knife cuts in the parasagittal plane of the hypothalamic fornix (KC)
4. intraventricular injection of 5,7-dihydroxytryptamine (5,7-DHT), a neurotoxin having its primary effect on neurons containing 5-hydroxytryptamine (serotonin)

5. ovariectomy.

Each of these procedures has been shown to cause overeating and greater than normal body weight gain (Hetherington & Ranson, 1940; Peters et al., 1979; Sclafani et al., 1976; Saller & Stricker, 1976; Zucker, 1969).

Three control groups were included in this experiment:

6. normal weight-restricted access (NWRA) -- this group was allowed unlimited food during the restricted access periods
7. 75% of normal weight (deprivation; DEP) -- this group was given restricted amounts of food during the access periods, such that it was limited to 75% of its presurgical body weight
8. free-feeding (FF) -- this group was allowed ad libitum access to food, and was never tested.

The three control groups each served a different function. The NWRA group was treated almost exactly as the experimental groups, and was their most appropriate control. The difference in treatment was due to the necessity of restricting the amount of food available to some animals in the experimental groups. These animals would have gained excessive amounts of weight if allowed unlimited food even for the relatively brief access periods. The FF group served as a control for any effects of a temporally restricted feeding schedule; by comparing the free-feeding group with

the normal weight-restricted access group, any changes in B-END levels due strictly to the feeding regimen could be made evident. The DEP group served as a control for any effect of continued "deprivation" of experimental animals relative to the elevated postsurgical weights they would have maintained if their food intakes were not restricted. Comparison of group DEP with group NWRA was intended to show any effects due to this relative deprivation of the experimental groups.

Testing sequence. Rats were divided into three squads for drug tests. Each surgical group was represented approximately equally in each squad. Each squad was tested every third day, allowing each squad two days between tests.

Each animal was tested once with each dose of naloxone HCl (0.5, 1.8, 6.8, and 25.0 mg/kg, ip), and received four saline tests. The lowest naloxone dose (0.5 mg/kg) is the lowest dose found effective in suppressing the food intake of lean rats by Margules et al. (1978). The highest dose (25.0 mg/kg) was chosen because preliminary work indicated lower doses (1.0 and 5.0 mg/kg) had little effect on feeding in normal animals in this laboratory. The two middle doses (1.8 and 6.8 mg/kg) divide the 0.5 to 25.0 mg/kg range into logarithmically equal intervals.

The total of eight tests for each rat was divided into four drug-saline pairs; that is, tests 1 and 2 constituted

one pair, tests 3 and 4 another pair, and so forth. This design was employed to allow appropriate analysis in the event that meaningful effects of repeated testing appeared. Whether the saline test paired with each naloxone test occurred on the first or second test day of each pair was random within the restrictions that: 1) on each test day approximately half of each surgical group was tested with saline, and half with naloxone; 2) no animal had exactly alternating naloxone and saline tests throughout the experiment (e.g. naloxone, saline, naloxone, saline, ...). Order of dose presentation was random within the restrictions that: 1) for no two rats in the same surgical group did two drug doses occur in the same order; 2) as far as possible, no two rats in the same surgical group were tested with the same dose on the same day.

Subjects

The subjects were female Long-Evans hooded rats (Blue Spruce Farms, Altamont, N.Y.) weighing 216 to 258 g at the beginning of the experiment. They were individually housed in standard hanging wire-mesh cages in a temperature controlled room (23 degrees C) under a 12/12 h light/dark cycle. During the dark portion of the cycle the room was illuminated by red light. Prior to surgery all animals had

free access to Teklad Mouse and Rat Pellets (Teklad, Winfield, Iowa).

Surgical procedures

Lesion, knife cut, and ovariectomized animals were anesthetized with 30 mg/kg sodium secobarbital ip (Myothesia, Beecham). Methyl scopolamine (10 mg/kg ip) was given to decrease respiratory secretion. Rats receiving intraventricular injections were lightly anesthetized with sodium secobarbital and brought to surgical anesthesia with ether. NWRA and DEP animals were lightly anesthetized with ether. All lesions were made using a stainless steel electrode insulated except for its 0.5 mm conical tip. Individual surgical procedures are described below.

VMH lesions. Bilateral VMH lesions were made with the tip of the electrode positioned 0.5 mm above the base of the brain, 5.8 mm anterior to the interaural line, and 0.7 mm lateral to the midline, with the incisor bar 5.0 mm above the interaural line. Lesions were produced by passing 2.0 mA anodal current for 20 s between the electrode and a rectal cathode.

DLT lesions. Bilateral lesions in the dorsolateral tegmentum were made with the incisor bar set 2.9 mm below the interaural line. The tip of the electrode was positioned 2.3 mm anterior to the interaural line, 1.5 mm lateral from the midline, and 3.0 mm above the interaural line. Lesions were

produced by passing 0.75 mA cathodal current for 20 s between the electrode and a rectal anode.

Knife cuts. Bilateral parasagittal cuts were made with a wire knife constructed after Hamilton and Timmons (1976) but not spring-loaded. The extended length of the wire blade was 3.0 mm. The incisor bar was set 5.0 mm above the interaural line. The knife, wire blade retracted, was lowered to approximately the level of the fornix (5.0 mm above the interaural line), 8.0 mm anterior to the interaural line and 1.0 mm lateral to the midline. The wire blade then was extended, the knife was lowered to the floor of the calvarium, and was raised to its original position. This down-and-up movement was repeated twice. The wire blade then was retracted, and the knife was withdrawn from the brain.

5,7-DHT injections. Intraventricular injections of 5,7-DHT consisted of 200 micrograms (free base) 5,7-DHT creatinine sulfate (Regis Chemical Company) dissolved in 0.1% ascorbic acid-normal saline vehicle. The injection volume of 20 microliters was delivered at 10 microliters per minute. The injection needle was left in place for one minute post-injection to allow for further diffusion of the neurotoxin. Injections were made with the needle tip positioned 0.5 mm posterior to bregma, 1.7 mm lateral to the midline, and 4.0 mm below the skull surface; the incisor bar was 5.0 mm above the interaural line. Desmethylimipramine

(Pertrofane, USV, 25 mg/kg ip) was injected 40 to 60 minutes prior to 5,7-DHT injection to diminish cytotoxic action in nonserotonergic neurons (Baumgarten, Klemm, Lachenmayer, Bjorklund, Lovenberg, & Schlossberger, 1978). Motor seizures, when they occurred, were controlled by giving additional sodium secobarbital to effect.

Ovariectomy. Ovaries were removed through bilateral dorsolateral incisions through the abdominal skin and muscle wall. A ligature was placed below the ovary before the ovary and a portion of the uterine horn were removed. The muscle wall was sutured closed and the skin was closed with wound clips.

Control surgery. Control surgery was performed on NWRA and DEP rats by lightly anesthetizing the animal, incising the scalp, and closing the wound.

Procedure

Post-surgical procedure. Prior to surgery all animals had free access to laboratory pellets. All operated animals were food and water deprived for 18 to 24 hours after surgery. FF rats, which were never operated upon, were deprived of food and water for 24 hours during the four-day surgical period. FF rats otherwise had ad libitum access to food and water throughout the experiment. The procedure outlined below is for all groups except FF.

After post-surgical deprivation each rat was given free access to food for 24 hours and to water for the remainder of the experiment. Following free access to food for 24 hours, each rat operated upon during the first three surgical days was placed on a feeding schedule allowing 24 hour access to only enough food to maintain the animal near its presurgical body weight. This procedure was employed to prevent hyperphagia and excessive weight gain. All rats then began a two-hour restricted access schedule. Each animal was allowed two hours access to enough food to maintain that animal at its presurgical body weight if all the food was consumed (except DEP rats; see below). Food remaining at the end of the two-hour period was removed from the cage. Rats operated upon on the fourth (last) surgical day went directly from 24-hour free access to two-hour restricted access. With the start of the two-hour restricted access feeding schedule, DEP rats were gradually reduced to 75% of their presurgical body weight by allowing them more limited amounts of food than available to other groups. After four days of two-hour restricted access, the duration of the access period was increased to four hours for all groups, and remained four hours throughout the testing period. All rats in the experimental groups were allowed, throughout the experiment, to weigh the same percentage of their presurgical body weight as the mean percentage of presurgical weight maintained by

the NWRA rats. As NWRA weights increased during the course of the experiment due to normal growth, the same percentage increases were allowed in the experimental groups. DEP rats were allowed 75% of these increases.

Drug testing. Naloxone effects on food intake were tested beginning 20 days after the last surgery. Daily procedure began with weighing all except FF rats. Following this the appropriate amount of food to maintain all rats not tested that day was determined. Appropriate injections then were given to the squad to be tested. Food was weighed for the test squad; once all food for this squad had been weighed it was delivered onto the cage floors, commencing the four-hour feeding period for this squad. The feeding period began approximately 15 minutes after the last injection. The food for the remaining two squads was then weighed and delivered. Four hours after the beginning of the test period food was withdrawn from the cages of the test squad; any food remaining in the cages of the squads not tested that day also was removed four hours after they had received their ration. Food intake to the nearest 0.1 g was determined for the test squad by subtracting the amount of food remaining at the end of the test period and spillage collected from under the cage from the amount of food delivered at the beginning of the test period. The four-hour feeding periods occurred over the fourth, fifth, sixth, and seventh hours of the dark period.

Beginning two days after the last naloxone test, all groups were divided in half such that the mean saline test intakes of each half was approximately the same, and the range of intakes in the entire group was reflected in each half as far as possible. One half of each group was randomly chosen to continue maintenance on the four-hour restricted access schedule. The other half of each group began ad libitum maintenance on a wet mash diet made of 70% tap water and 30% Wayne Lab Blox. The wet mash diet was employed to promote weight gain and the development of hyperphagia (e.g. Peters, Wellman, & Gunion, 1979). Wet mash was delivered fresh every 24 hours in glass ointment jars (81 mm high by 70 mm wide) placed in the cage, and held to the cage front by a wire loop. Body weight and 24-hour food intake of the rats fed the wet-mash diet were measured every three days for 36 days. The wet mash given on day 11 was prepared with too much water. For this reason food intake on day 12 was not measured.

Beginning 33 days after the last naloxone test, animals maintained on the restricted access feeding schedule were sacrificed. Animals fed the wet mash diets were sacrificed beginning 39 days after the last naloxone test.

Beta-endorphin assay

The rats were sacrificed by decapitation. Operated brains were removed and saved in 10% Formalin for histology.

Pituitaries were removed, gently blotted with a laboratory wipe, and weighed to the nearest 0.1 mg. Pituitaries were then placed in separate 50 ml polyethylene (Nalgene) centrifuge tubes containing 2.0 ml of hot 1 N acetic acid. The tubes were immersed in a boiling water bath. After 15 minutes the tubes were removed from the bath and chilled in ice; when cold the tissue was ground in the same tube using a motor-driven Teflon pestle. The tube was allowed to stand in ice until centrifuged at 1200 x g for 50 to 70 minutes. The clear supernatant was saved and stored at -15 degrees C.

The frozen supernatant was freeze-dried for 48 hours and stored at -15 degrees C until assay. The extract was prepared for assay by reconstitution with 2.0 ml of cold buffer (0.1 M phosphate at pH 6.0, 0.005 M EDTA, 0.05 M NaCl, 5.0% mannitol, 0.1% gelatin, and 0.005% merthiolate). The tube was vortexed and allowed to stand on ice for 15 minutes. The extract was not completely solubilized, and the tube was centrifuged 15 minutes at 800 x g. One-tenth ml of the clear supernatant was used for assay of B-END content. B-END content was measured with a commercially available radioimmunoassay kit (New England Nuclear, Boston, NEK-003). The buffer used for reconstitution duplicated that supplied with the assay kit.

Histology

Brains saved at sacrifice were stored in 10% Formalin until frozen sections (100 microns) were taken in the coronal plane. Photographic enlargements of these unstained sections were used to assess the location and extent of tissue damage, with reference to the atlas of Konig and Klippel (1963).

Statistical analysis

The effects of naloxone on food intake were analyzed both as intake in grams and as percentage decrease from the mean of the saline control tests. Analysis of variance (ANOVA) was followed by a posteriori evaluation using the Duncan New Multiple Range Test (Kirk, 1968) where warranted. Food intake and body weight data of rats fed the wet mash diet were also analyzed using ANOVAs followed by the Duncan New Multiple Range Test. All rats had a missing value for food intake on day 12 of wet mash consumption. Initial analysis of the assay data was done by regression, and was followed by the Duncan New Multiple Range Test.

Results

Histology

Examples of the destruction caused by VMH lesions, hypothalamic knife cuts, DLT lesions, and 5,7-DHT injections are shown in Figure 1.

VMH lesions. (Figure 1a). VMH lesions generally destroyed the area posterior to the anterior nucleus, rostral to the premammillary nuclei, ventral to the dorsomedial nucleus, and medial to the fornix. There was a strong tendency for the lesions to include the premammillary nuclei and heavily damage the mammillary bodies as well. In most cases there was little or no damage to the anterior nucleus. The lesion usually spread laterally to include the fornix within its border, and often, but not always, spread slightly lateral to the fornix. Four rats were discarded from this group for failure to meet the preceding description; in general, their lesions were quite posterior and spared much of the rostral portions of the VMH.

Knife cuts. (Figure 1b). The cuts began rostrally at the level of the optic chiasm. At this level, the cuts extended vertically from the fornix to the optic chiasm or, in a few cases, through the lateral portion of the optic chiasm. At the caudal edge of the anterior nucleus the cuts extended from the fornix to the base of the brain. This vertical plane encompassed approximately the rostral

Figure 1. Photomicrographs of representative sections showing damage caused by:

(a) ventromedial hypothalamic lesions

(b) hypothalamic knife cuts (arrows point to cuts)

(c) dorsolateral tegmental lesions

(d) intraventricular 5,7-dihydroxytryptamine (arrow points to injection needle track).

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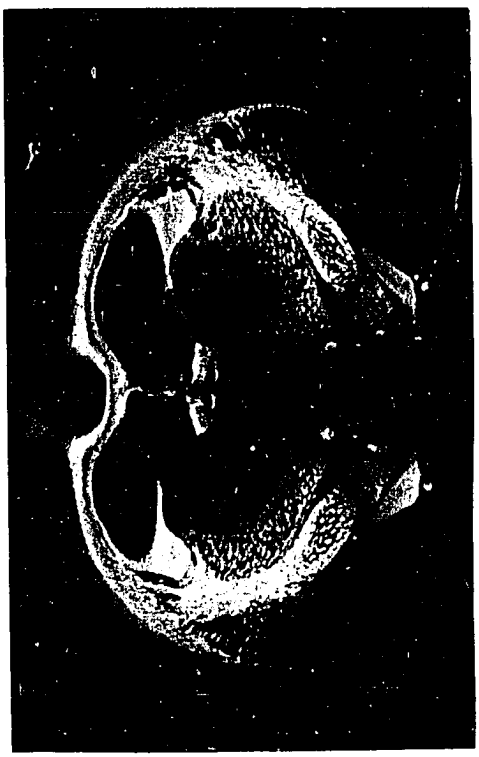

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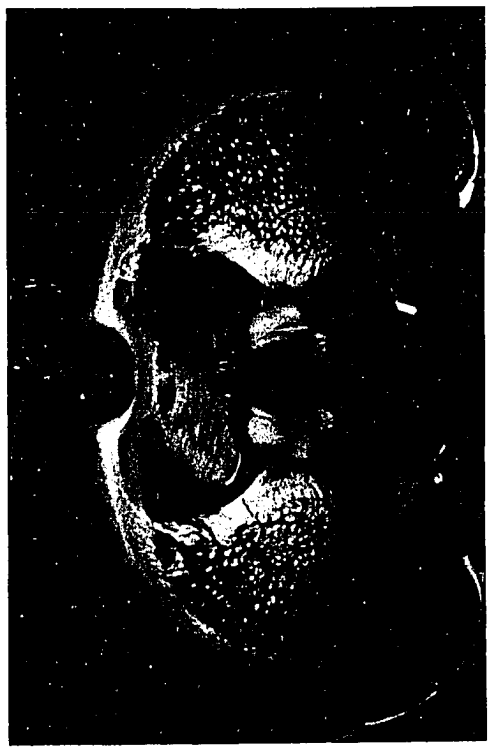

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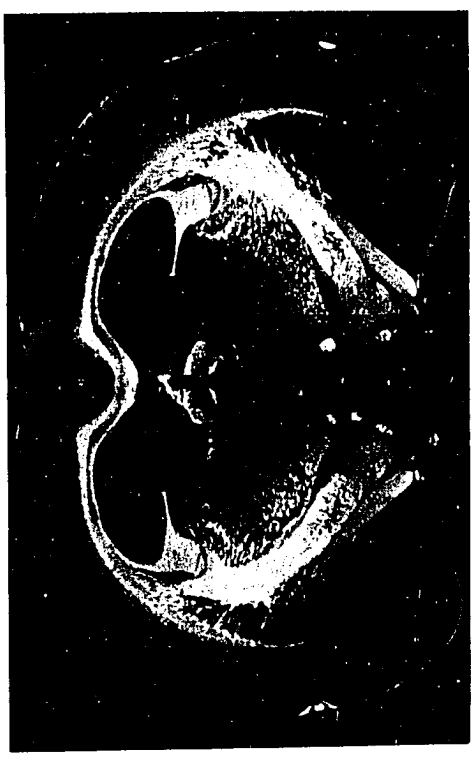

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c

two-thirds of the ventromedial nucleus (VMN); at this point both the dorsal and ventral extent of the cut began to rise. Cuts typically terminated in the premammillary nuclei. At this level the cuts extended between the mammillothalamic tract and the fornix. The cuts lay in or very near the parasagittal plane of the fornix; several times the wire blade had been deflected by the fornix. The third ventricle often was slightly enlarged. Three rats were discarded from this group. Two had substantially enlarged ventricles, and one had a cut that did not reach the base of the brain at the level of the rostral VMN.

DLT lesions. (Figure 1c). The location and extent of the DLT lesions were as recently described (Peters, Gunion, & Wellman, 1979). These cathodal lesions appear as thin vertical slits with dorsal extents adjacent to the ventrolateral edge of the periaqueductal gray, and ventral extents to the level of the superior cerebellar peduncle. Rostrally, the lesions begin within the exiting fibers of the oculomotor nerve and continue caudally as far as the decussation of the superior cerebellar peduncle. One rat was discarded because one of its lesions was too lateral.

5,7-DHT injections. (Figure 1d). A discernible injection needle track through the cortex and into the lateral ventricle was generally visible. Some damage to the dorsal hippocampus was evident in a number of animals. All

animals but one showed vibrisseal vibration at injection, and in many animals the pinnae folded caudally, flat against the head. These effects began approximately 30 seconds after the end of injection. One animal was discarded from this group. This animal did not show vibration of the vibrissae at injection, and the ventricle receiving the injection was grossly enlarged. The absence of behavioral signs and the enlarged ventricle suggest that, for some unknown reason, the injection volume may not have immediately passed out of the lateral ventricle.

Final group size. In addition to loss by histology, two animals died after surgery. One animal from group 5,7-DHT died on the day after surgery shortly after access to food and water. It appeared healthy when food and water were delivered. One animal from group OVX died several weeks after surgery for unknown reasons.

Final group sizes were 13 for DLT, 10 for 5,7-DHT, 11 for KC, 11 for OVX, 12 for DEP, 12 for NWRA, 12 for FF, and 10 for VMH.

Naloxone effects on food intake

Analyses of variance (ANOVAs) first were performed on intakes during the four saline tests to examine the effects of repeated testing. One between factor (Group) and one within factor (Test) were analyzed. As expected, Group was significant ($F_{6, 72} = 7.60$; $p < .0001$), but Test was not (F

3, 216 = 1.30; p = .275). In a further examination for effects of previous testing, the first saline test data were split into two groups: those animals for which the first of the eight total tests was saline, and those animals for which the first saline test was preceded by a naloxone test. Again, Group was significant (F 6, 65 = 6.17; p < .0001) while Test was not (F 1, 65 = 0.52; p = .474). It was concluded that there was no effect of saline test order. The four saline test values were averaged to obtain one value for the saline value that was used in all subsequent analyses.

Naloxone effects were analyzed both as grams intake and as percentage change in intake. When analyzed as grams intake, both Group (F 6, 72 = 7.70; p < .0001; Figure 2a) and Dose (F 4, 287 = 55.19; p < .0001; Figure 2b) were significant. The Group x Dose interaction, however, was not significant (F 24, 287 = 1.18; p = .259; Figure 3a). Thus, while both naloxone dose and surgical group had significant effects on food intake, no surgical group was more or less sensitive to the effects of naloxone on food intake than any other.

Table 1 and Figure 2a show the mean food intake of each surgical group averaged across dose. Duncan's New Multiple Range Test (Kirk, 1968) was used to compare mean intakes of the groups. Groups KC, 5,7-DHT, DLT, and VMH ate significantly more than group NWRA (all p < .01), while

Table 1. Surgical group mean intakes ± S.E. (g). See text for group abbreviations. Results of statistical comparisons are shown beneath the data: numbers are p values, ns means not significant.

Group	Intake (g)
KC	17.1 ± 0.3
5,7-DHT	16.0 ± 0.5
DLT	15.9 ± 0.4
VMH	15.6 ± 0.6
OVX	13.5 ± 0.3
DEP	12.5 ± 0.4
NWRA	12.0 ± 0.3

Comparisons

KC versus		VMH versus	
5,7-DHT	ns	OVX	ns
DLT	ns	DEP	.01
VMH	ns	NWRA	.01
OVX	.01		
DEP	.01		
NWRA	.01		
5,7-DHT versus		OVX versus	
DLT	ns	DEP	ns
VMH	ns	NWRA	ns
OVX	ns		
DEP	.01		
NWRA	.01		
DLT versus		DEP versus	
VMH	ns	NWRA	ns
OVX	ns		
DEP	.01		
NWRA	.01		

Figure 2. Food intakes during naloxone testing.

(a) Main effect of surgical group on grams of food consumed during testing. Groups (see pp 9-10 for further explanation):

DEP -- deprived to 75% of normal weight

DLT -- dorsolateral tegmental lesion

5,7-DHT -- intraventricular 5,7-dihydroxytryptamine

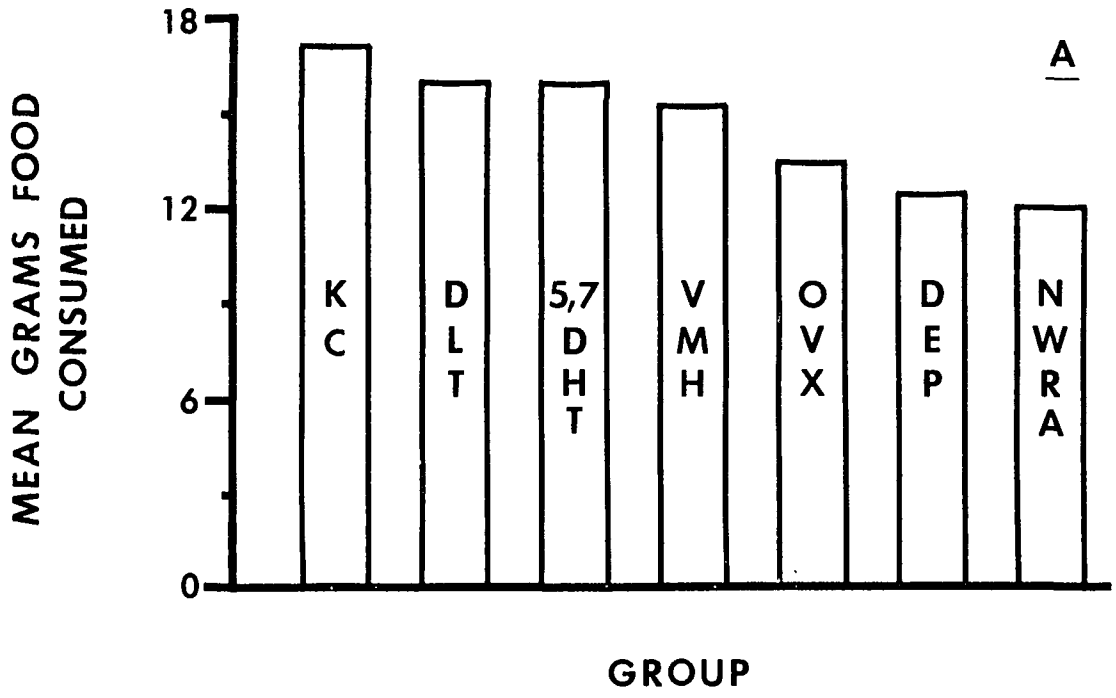
KC -- hypothalamic knife cut

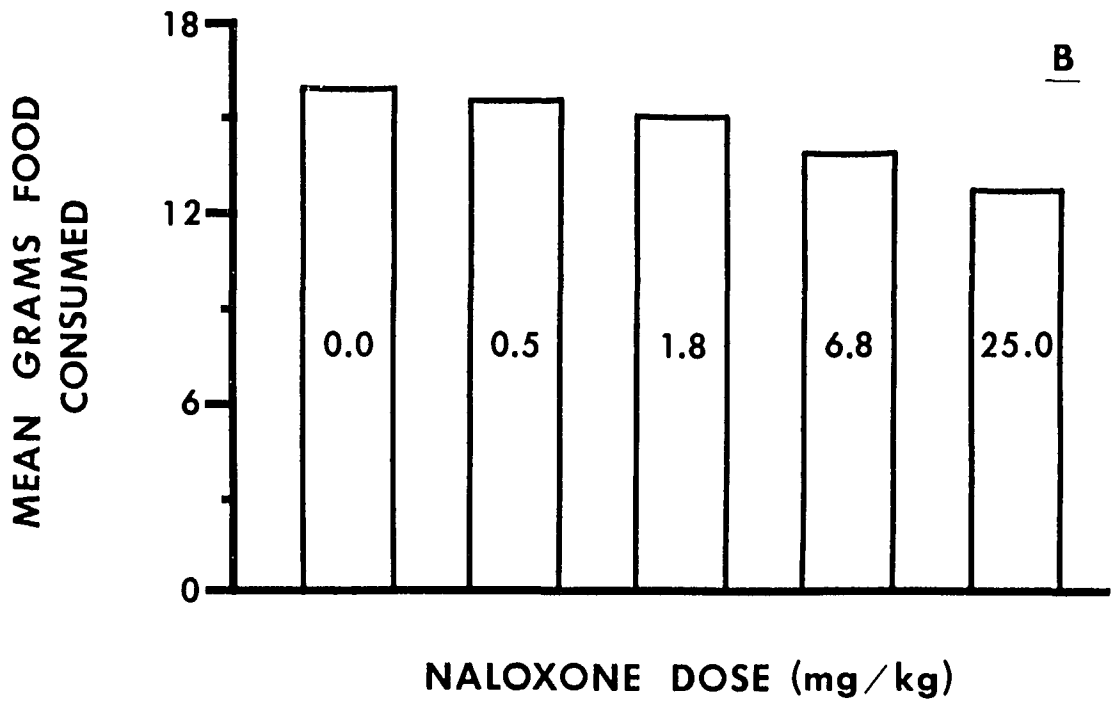
NWRA -- normal weight, restricted access

OVX -- ovariectomy

VMH -- ventromedial hypothalamic lesion.

(b) Main effect of naloxone dose on grams of food consumed during testing.


A
MEAN GRAMS FOOD CONSUMED
18
12
6
0
KC
DLT
5,7 DHT
VMH
OVX
DEP
NWRA
GROUP


B
MEAN GRAMS FOOD CONSUMED
18
12
6
0
0.0
0.5
1.8
6.8
25.0
NALOXONE DOSE (mg/kg)

groups OVX and DEP did not (both $p > .05$). Groups KC, 5,7-DHT, DLT, and VMH ate significantly more than DEP (all $p < .01$), but only group KC ate significantly more than OVX ($p < .01$; all others $p > .05$). Groups KC, 5,7-DHT, DLT, and VMH did not eat significantly different amounts (all $p > .05$). Table 2 and Figure 2b show the mean intake at each dose of naloxone, averaged across group. Doses of 1.8, 6.8, and 25.0 mg/kg significantly decreased intake (all $p < .01$); only 0.5 mg/kg did not ($p > .05$). Further, each of the three effective doses caused significantly greater suppression than the next lower dose ($p < .05$ for 0.5 versus 1.8; others, $p < .01$).

When the data were analyzed as percentage change in intake, Group was not significant ($F\ 6, 72 = 1.24$; $p = .627$). Dose was significant ($F\ 4, 287 = 56.08$; $p < .0001$; Table 3), while the Group x Dose interaction was not ($F\ 24, 287 = 1.26$; $p = .299$; Figure 3b). Table 3 shows the mean percentage of saline intake at each dose. Comparisons among means showed exactly the same differences to be significant and nonsignificant as were found when the data were analyzed simply as mean grams intake. Significance levels also were unaltered.

Table 2. Mean intake ± S. E. at each dose of naloxone. Results of statistical comparisons are shown beneath the data: numbers are p values, ns means not significant.

Dose (mg/kg)	Intake (g)
0.0	15.9 ± 0.3
0.5	15.6 ± 0.4
1.8	15.0 ± 0.4
6.8	13.8 ± 0.4
25.0	12.7 ± 0.4

Comparisons

0.0 versus		1.8 versus	
0.5	ns	6.8	.01
1.8	.01	25.0	.01
6.8	.01		
25.0	.01		
0.5 versus		6.8 versus	
1.8	.05	25.0	.01
6.8	.01		
25.0	.01		

Figure 3. Effect of naloxone dose on food consumption during testing:

(a) in grams of food consumed

(b) as a percentage of the amount of food consumed during saline (0.0 mg/kg) tests.

Groups (see pp 9-10 for further explanation):

- ● DEP -- deprived to 75% of normal weight
- ▫ DLT -- dorsolateral tegmental lesion
- ■ 5,7-DHT -- intraventricular 5,7-dihydroxytryptamine
- ▼ KC -- hypothalamic knife cut
- X NWRA -- normal weight, restricted access
- o OVX -- ovariectomy
- ▽ VMH -- ventromedial hypothalamic lesion

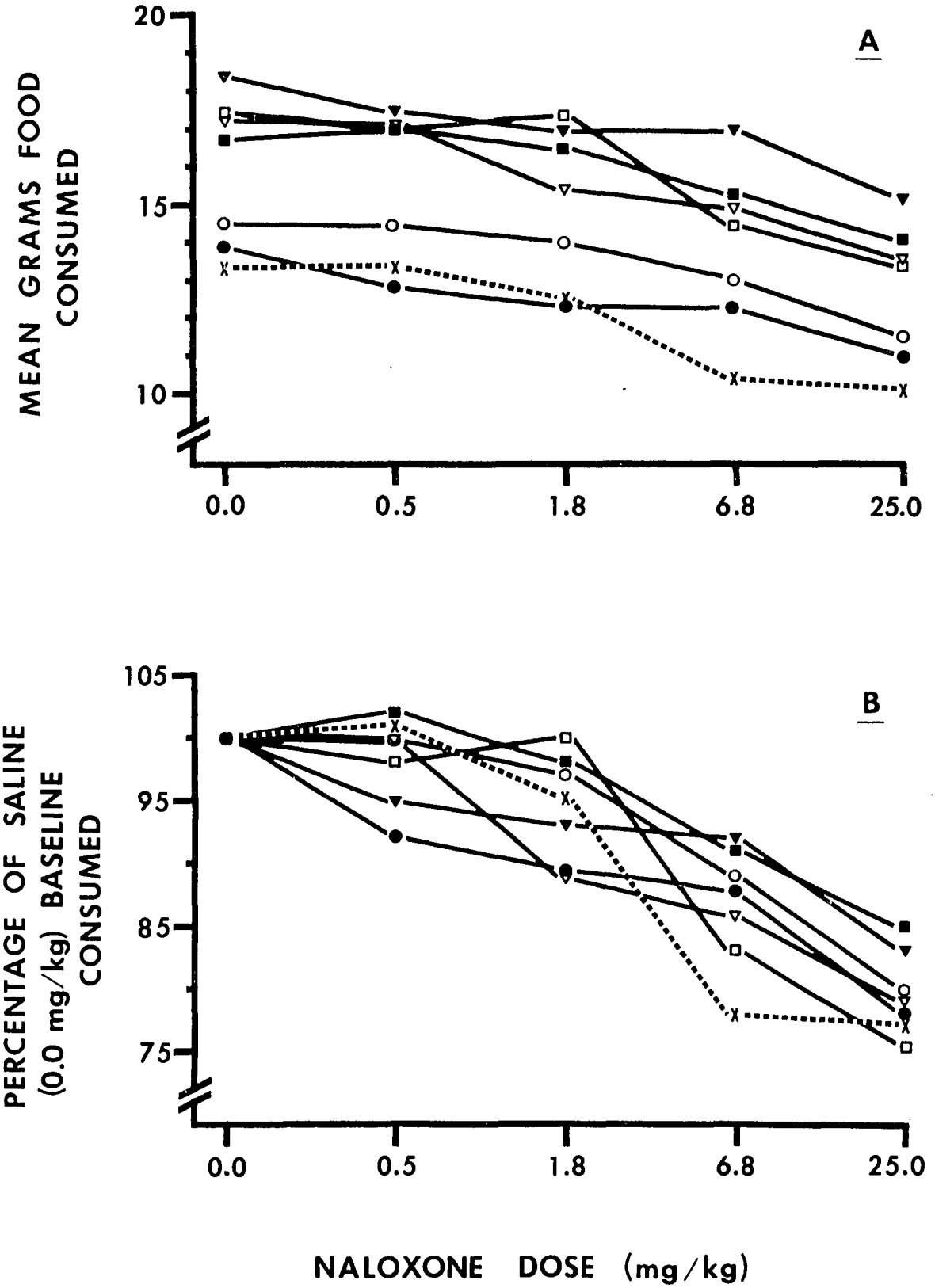

A
MEAN GRAMS FOOD CONSUMED
20
15
10
0.0
0.5
1.8
6.8
25.0
B
PERCENTAGE OF SALINE (0.0 mg/kg) BASELINE CONSUMED
105
95
85
75
0.0
0.5
1.8
6.8
25.0
NALOXONE DOSE (mg/kg)

Table 3. Percent of saline test intake ± S. E. at each dose of naloxone. Results of statistical comparisons are shown beneath the data: numbers are p values, ns means not significant.

Dose (mg/kg)	Percentage Intake
0.0	100.0 ± 0.0
0.5	98.2 ± 1.5
1.8	94.4 ± 1.4
6.8	86.4 ± 1.5
25.0	79.6 ± 1.2

Comparisons

0.0 versus		1.8 versus	
0.5	ns	6.8	.01
1.8	.01	25.0	.01
6.8	.01		
25.0	.01		
0.5 versus		6.8 versus	
1.8	.05	25.0	.01
6.8	.01		
25.0	.01		

Beta-endorphin assay

The manufacturer of the assay kit (New England Nuclear) states that the kit B-END antibody has 100% cross-reactivity with B-END and 50% cross-reactivity with beta-lipotropin. Alpha-endorphin, leu-enkephalin, met-enkephalin, alpha-melanocyte stimulating hormone, and adrenocorticotropic hormone are said to show less than 0.01% cross-reactivity to this antibody. Bloom, Rossier, Battenberg, Bayon, French, Henriksen, Siggins, Segal, Browne, Ling, and Guillemin (1978) have reported that, using gel chromatography and an antibody having 100% cross-reactivity to both B-END and beta-lipotropin, approximately 67% of the total immunoreactivity of rat whole pituitary extract can be attributed to B-END and the remaining 33% to beta-lipotropin. It follows, then, that the total B-END-like immunoreactivity found by the assay kit in rat whole pituitary extract should be approximately 83% B-END and 17% beta-lipotropin. B-END concentrations determined in this experiment, then, reflect a contribution from beta-lipotropin. To avoid confusion, values are expressed here as picograms of beta-endorphin-like immunoreactivity (B-ENDLI) per milligram of wet pituitary tissue.

All samples assayed on the first day, as well as the standard curve, were lost due to the addition of insufficient amounts of tracer. This left the DEP-wet mash cell with an n

of two, the FF-pellet cell with an n of three, and the NWRA-pellet cell with an n of four. All other cells had five to seven animals each.

Additional total counts, blank, and zero standard tubes were run and values from these tubes used to determine the normalized percent bound for the remaining samples. The "technical data" standard curve supplied with the kit was used to determine the B-ENDLI content of the samples. This approach was considered acceptable, since previous work with these kits had repeatedly produced standard curves highly similar to those supplied. B-ENDLI content of samples determined in this manner were well in line with previous work using these kits. Using this method, sample aliquots diluted to half concentration before assay showed B-ENDLI contents half those of the undiluted samples.

Because of loss of some samples at assay, cell n's were too unequal for ANOVA. Consequently, regression (partial sums of squares) was used to analyze the B-ENDLI concentration (pg/mg wet weight) data. The main effect of Group was significant ($F_{7, 60} = 5.08$; $p < .0003$; Table 4; Figure 4a), as was the main effect of Diet ($F_{1, 60} = 4.63$; $p < .03$; Figure 4b). The mean values of B-ENDLI ranged from 23.1 pg/mg to 71.6 pg/mg among the surgical groups. For the two diets the means were 51.0 pg/mg for animals sacrificed at restricted weight (pellet diet), and 58.7 pg/mg for animals

Table 4. Surgical group mean ± S. E. concentrations of pituitary beta-endorphin-like immunoreactivity (pg/mg wet weight). See text for group abbreviations. Results of statistical comparisons are shown beneath the data: numbers are p values, ns means not significant.

Group	B-ENDLI (pg/mg)
DEP	71.6 ± 4.9
DLT	63.0 ± 7.1
VMH	59.6 ± 7.9
NWRA	59.5 ± 7.3
OVX	56.0 ± 7.5
FF	55.4 ± 7.8
5,7-DHT	47.9 ± 3.5
KC	23.1 ± 3.5

Comparisons

DEP versus		NWRA versus	
DLT	ns	OVX	ns
VMH	ns	FF	ns
NWRA	ns	5,7-DHT	ns
OVX	ns	KC	.01
FF	ns		
5,7-DHT	.05		
KC	.01		
		OVX versus	
DLT versus		FF	ns
VMH	ns	5,7-DHT	ns
NWRA	ns	KC	.01
OVX	ns		
FF	ns		
5,7-DHT	ns		
KC	.01	FF versus	
		5,7-DHT	ns
VMH versus		KC	.01
NWRA	ns		
OVX	ns		
FF	ns		
5,7-DHT	ns	5,7-DHT versus	
KC	.01	KC	.01

Figure 4. Pituitary beta-endorphin-like immunoreactivity concentrations.

(a) Main effect of surgical group on concentration of pituitary beta-endorphin-like immunoreactivity (pg/mg).

Groups (see pp 9-10 for further explanation):

DEP -- deprived to 75% of normal weight

DLT -- dorsolateral tegmental lesion

5,7-DHT -- intraventricular 5,7-dihydroxytryptamine

FF -- free feeding

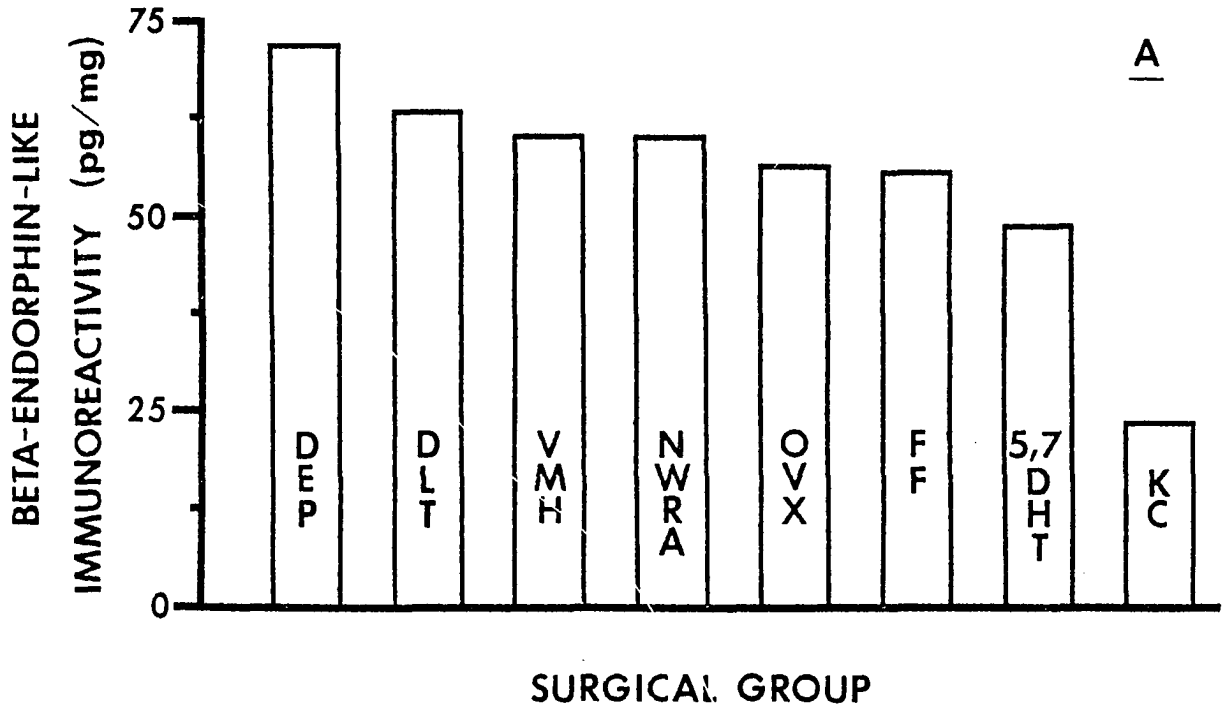
KC -- hypothalamic knife cut

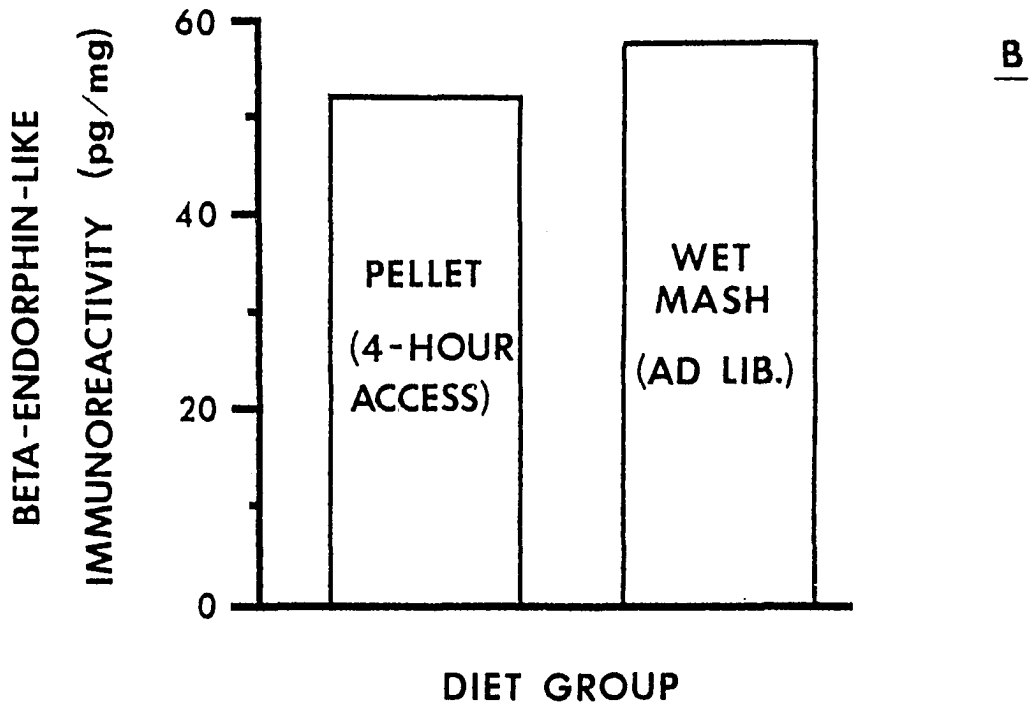
NWRA -- normal weight, restricted access

OVX -- ovariectomy

VMH -- ventromedial hypothalamic lesion.

(b) Main effect of feeding regimen at sacrifice on concentration of pituitary beta-endorphin-like immunoreactivity (pg/mg). Pellet (4-hour access) rats were sacrificed at normal body weight. Wet mash (ad lib.) rats were sacrificed at free-feeding body weight.


A
BETA-ENDORPHIN-LIKE IMMUNOREACTIVITY (pg/mg)
75
50
25
0
DEP
DLT
VMH
NWRA
OVX
FF
5,7 DHT
KC
SURGICAL GROUP


B
BETA-ENDORPHIN-LIKE IMMUNOREACTIVITY (pg/mg)
60
40
20
0
PELLET
(4-HOUR ACCESS)
WET MASH
(AD LIB.)
DIET GROUP

sacrificed at ad libitum weight (wet mash diet). The Group x Diet interaction was not significant ($F_{7, 60} = 0.76$; $p = .627$).

Duncan's New Multiple Range Test (Kirk, 1968) was used to further examine the effect of Group on B-ENDLI concentrations (Table 4). The concentration of B-ENDLI for group KC (23.1 mg/kg) was significantly less than that of each of the other groups (mean of 59.8 pg/mg across these groups; all $p < .01$). Additionally, 5,7-DHT (47.9 pg/mg) had significantly less B-ENDLI than DEP (71.6 pg/mg; $p < .05$). No other differences were significant.

The significantly lower concentration of pituitary B-ENDLI in 5,7-DHT rats when compared to DEP rats is puzzling. This difference does not appear to have any relationship with the general pattern of results of this work. Neither DEP nor 5,7-DHT groups differed significantly from the NWRA or FF control groups. It should be considered here that the difference between DEP and 5,7-DHT was significant only at the .05 level; in fact, had the mean difference of 23.7 pg/mg been just 3.2 pg/mg less, the difference would not have been significant at all. At present the significant difference in pituitary B-ENDLI concentrations between groups 5,7-DHT and DEP seems most reasonably interpreted as Type I (alpha) error, and it will not be further discussed.

Figure 5. Group mean body weights of animals allowed ad libitum access to the wet mash diet after the end of naloxone testing. Group ns are shown in the key.

Groups (see pp 9-10 for further explanation):

DEP -- deprived to 75% of normal weight

DLT -- dorsolateral tegmental lesion

5,7-DHT -- intraventricular 5,7-dihydroxytryptamine

FF -- free feeding

KC -- hypothalamic knife cut

NWRA -- normal weight, restricted access

OVX -- ovariectomy

VMH -- ventromedial hypothalamic lesion.

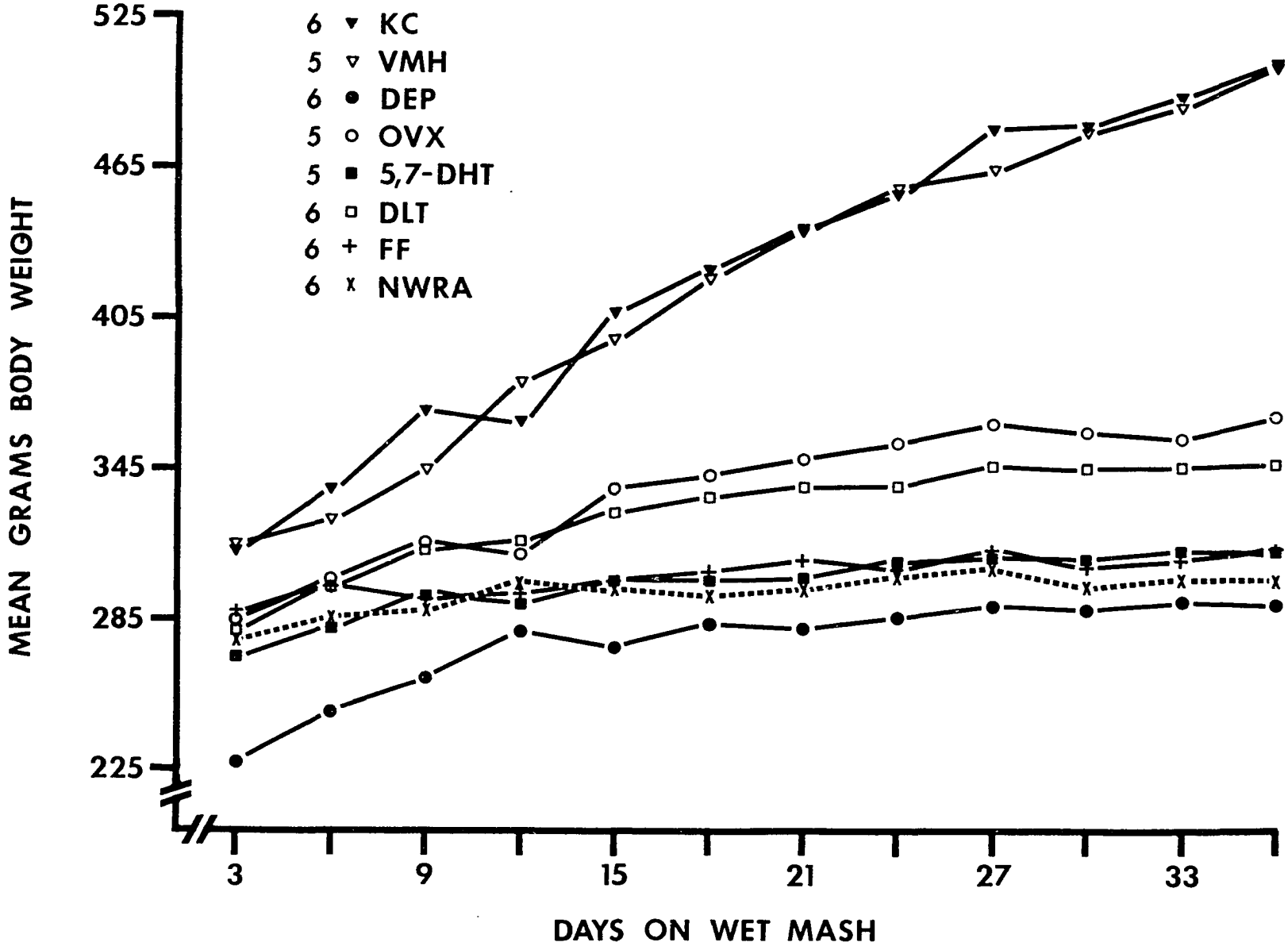

6 KC
5 VMH
6 DEP
5 OVX
5 5,7-DHT
6 DLT
6 FF
6 NWRA
525
465
405
345
285
225
MEAN GRAMS BODY WEIGHT
3
9
15
21
27
33
DAYS ON WET MASH

Figure 5 shows the changes in body weight that occurred during the first 36 days ad libitum access to the wet mash diet. ANOVA showed significant effects of Group ($F_{7, 37} = 27.78$; $p < .0001$), Day ($F_{11, 407} = 458.77$; $p < .0001$), and Group x Day ($F_{77, 407} = 45.81$; $p < .0001$). Analyses of group mean body weights on day 36 revealed that body weights formed three distinct clusters. A "very obese" cluster consisted of groups VMH and KC; a "moderately obese" cluster consisted of groups DLT and OVX; and a "normal weight" cluster consisted of groups 5,7-DHT, NWRA, DEP, and FF. Within each cluster, no group weighed significantly more or less than any other group (all $p > .05$). All other comparisons (that is, those made between groups from different clusters) were significant (DLT versus 5,7-DH, $p < .05$; DLT versus FF, $p < .05$; all others, $p < .01$); see Table 5).

The food intake data paralleled the body weight data (see Figure 6). Again, ANOVA showed significant effects of Group ($F_{7, 37} = 53.83$; $p < .001$), Day ($F_{10, 370} = 36.73$; $p < .0001$), and Group x Day ($F_{70, 370} = 9.13$; $p < .0001$). The main effect of surgical group on wet mash consumption is shown in Table 6. All groups (KC, VMH, OVX, DLT) which weighed significantly more than group NWRA on day 36 also ate significantly more than NWRA over the 36 day period (OVX versus NWRA, $p < .05$; all others, $p < .01$). Groups (5,7-DHT,

DEP, FF) that did not have weights significantly different from NWRA on day 36 did not eat amounts significantly different from NWRA (all $p > .05$).

Table 5. Group mean body weights ± S. E. (g) on day 36. See text for abbreviations. Results of statistical comparisons are shown beneath the data: numbers are p values, ns means not significant.

Group	Body Weight (g)
KC	505.8 ± 11.6
VMH	505.0 ± 44.5
OVX	365.8 ± 16.7
DLT	347.8 ± 10.0
FF	312.0 ± 21.7
5,7-DHT	311.6 ± 8.5
NWRA	301.0 ± 17.6
DEP	291.5 ± 9.3

Comparisons

KC versus		DLT versus	
VMH	ns	FF	.05
OVX	.01	5,7-DHT	.05
DLT	.01	NWRA	.01
FF	.01	DEP	.01
5,7-DHT	.01		
NWRA	.01		
DEP	.01		
		FF versus	
VMH versus		5,7-DHT	ns
OVX	.01	NWRA	ns
DLT	.01	DEP	ns
FF	.01		
5,7-DHT	.01		
NWRA	.01		
DEP	.01	5,7-DHT versus	
		NWRA	ns
OVX versus		DEP	ns
DLT	ns		
FF	.01		
5,7-DHT	.01		
NWRA	.01	NWRA versus	
DEP	.01	DEP	ns

Figure 6. Group mean food intakes of animals allowed ad libitum access to the wet mash diet after the end of naloxone testing. Group ns are shown in the key.

Groups (see pp 9-10 for further explanation):

DEP -- deprived to 75% of normal weight

DLT -- dorsolateral tegmental lesion

5,7-DHT -- intraventricular 5,7-dihydroxytryptamine

FF -- free feeding

KC -- hypothalamic knife cut

NWRA -- normal weight, restricted access

OVX -- ovariectomy

VMH -- ventromedial hypothalamic lesion.

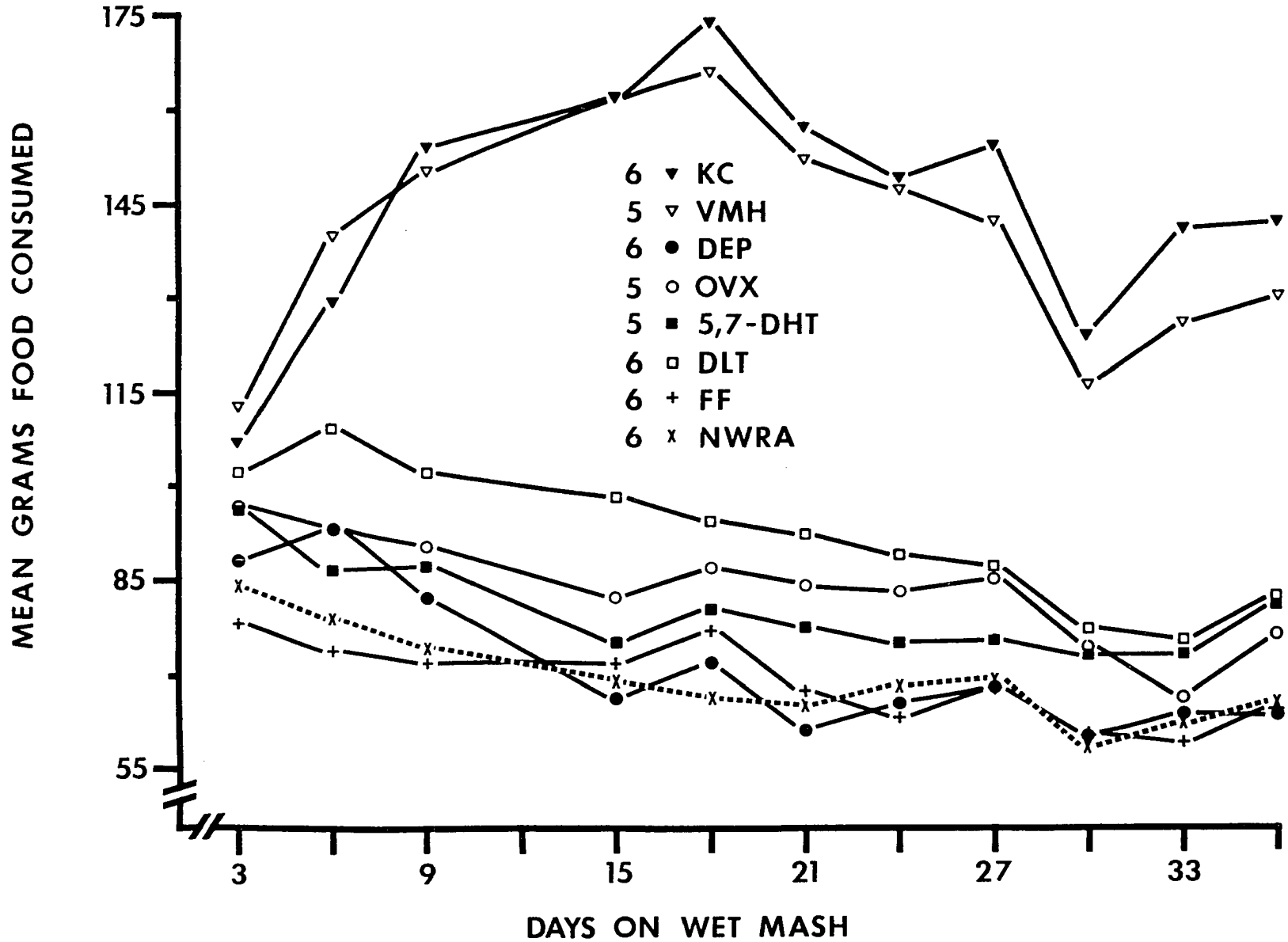

MEAN GRAMS FOOD CONSUMED
175
145
115
85
55
3
9
15
21
27
33
DAYS ON WET MASH
6 ▼ KC
5 ▽ VMH
6 ● DEP
5 ○ OVX
5 ■ 5,7-DHT
6 □ DLT
6 + FF
6 x NWRA

Table 6. Main effect of group on 24-hour wet mash food intakes ± S. E. (g). See text for abbreviations. Results of statistical comparisons are shown beneath the data: numbers are p values, ns means not significant.

Group	Food Intake (g)
KC	144.6 ± 2.5
VMH	140.3 ± 2.9
DLT	90.9 ± 2.3
OVX	83.4 ± 1.8
5,7-DHT	79.9 ± 1.4
DEP	70.8 ± 1.7
NWRA	68.9 ± 2.1
FF	68.6 ± 1.3

Comparisons

KC versus		OVX versus	
VMH	ns	5,7-DHT	ns
DLT	.01	DEP	ns
OVX	.01	NWRA	.05
5,7-DHT	.01	FF	.05
DEP	.01		
NWRA	.01		
FF	.01		
		5,7-DHT versus	
VMH versus		DEP	ns
DLT	.01	NWRA	ns
OVX	.01	FF	ns
5,7-DHT	.01		
DEP	.01		
NWRA	.01		
FF	.01	DEP versus	
		NWRA	ns
DLT versus		FF	ns
OVX	ns		
5,7-DHT	ns		
DEP	.01		
NWRA	.01	NWRA versus	
FF	.01	FF	ns

Discussion

Effects of naloxone on food intake

Naloxone decreased food intake in a dose-related manner. The effect of naloxone was not different among the groups; that is, the feeding behavior of no group was more or less affected by naloxone than was the feeding behavior of the control (NWRA) group.

Three possible reasons for the failure to find a differential effect of naloxone on feeding among the surgical groups are immediately apparent. The first, of course, is that no such effect may actually exist for the surgical treatments included in this experiment. This may indeed be the case, although one experiment reporting negative results is not a strong confirmation of this possibility.

A second possibility is that the doses of naloxone used (0.5, 1.8, 6.8, and 25.0 mg/kg) are not doses which would show this effect, and that lower or higher doses are required. There are reasons to think, however, that this is not the case. First, the lowest dose of naloxone used in this experiment did not cause a significant reduction in food intake for any group; the possibility that the lowest dose was too large to show differential effects on groups must therefore be rejected. It is possible that the largest dose (25.0 mg/kg) was not large enough to show group differences in naloxone sensitivity. This argument is countered,

however, by the fact that 25.0 mg/kg is the largest dose of naloxone that has been used to suppress feeding in any reported experiment. Further, this dose of naloxone may inhibit feeding, at least in part, by producing an aversive state within the animal. Since the present work was begun, Frenk and Rogers (1979) have reported that a single injection of 10 mg/kg (ip) naloxone can be used to establish a conditioned taste aversion in the rat. The 25.0 mg/kg dose of naloxone used here, then, is greater than the range in which purely specific effects of naloxone on feeding can be observed. The doses Margules et al. (1978) used to obtain greater than control suppression of feeding in genetically obese rats were 0.5 and 1.0 mg/kg; doses higher than 1.0 mg/kg were not used. Given the known range of effective doses (0.5 to 1.0 mg/kg), it is conceivable that the doses used in this experiment might fall entirely outside the effective range (i.e., 0.5 was too low in this case, and 1.8 too high). While conceivable, however, such an explanation is not appealing, since it would require any differential effect to appear and disappear within a narrow dose range.

The third possible reason for the failure to find differential effects of naloxone among the surgical treatments in this experiment may lie in the body composition of the animals. Margules et al. (1978) made no attempt to control the body weights of the genetically obese mice and

rats used in their work. The genetically obese rats weighed 68% to 178% more than did their lean littermate controls. In the present experiment all rats (except DEP) were maintained at the same percentage of their presurgical body weights. If the animals used in the present experiment had been allowed to gain weight freely, differential effects of naloxone on feeding might have been found. This possibility will be further discussed below.

A key question in evaluating the role of endogenous opioids in the regulation of feeding behavior is whether the effect of naloxone in suppressing food intake is central, peripheral, or perhaps both central and peripheral. A basis exists for naloxone suppression of feeding by a peripheral mechanism. Ipp et al. (1978) have shown that B-END causes insulin release from pancreatic islets in vitro; this effect is blocked by naloxone. A number of studies have shown that exogenous insulin can stimulate food intake and induce obesity (e.g. Hoebel & Teitelbaum, 1966). Naloxone, then, could block or depress B-END-stimulated insulin release at the pancreas, thus inhibiting a known stimulus to feeding and obesity development.

A reasonable basis for naloxone suppression of feeding by central mechanisms also exists. Both intrahypothalamic (Grandison & Guidotti, 1977) and intraventricular (Kenny et al., 1978) administration of B-END elicit feeding. The

feeding effect of intrahypothalamic B-END is blocked by naloxone. (Naloxone effects on feeding behavior induced by intraventricular administration of B-END have not been tested). Further, B-END is found in brain areas associated with feeding, including the medial and ventral hypothalamus (Bloom, Rossier, Battenberg, Bayon, French, Henriksen, Siggins, Segal, Browne, Ling, & Guillemin, 1978). These findings suggest that central B-END feeding mechanisms may exist. Systemically administered naloxone could suppress feeding by antagonizing central B-END mechanisms involved in feeding.

A complicating factor (as if one were needed) is that pituitary hormones, including B-END, may be directly transported from the pituitary to the brain by the pituitary venous drainage (Bergland & Page, 1979). Presumably, these hormones would have effects in the brain. Peripherally released (pituitary) B-END, then, could affect feeding by both central and peripheral mechanisms. Either or both of these mechanisms might be synergistic, antagonistic, or unrelated to the feeding effects of centrally released B-END. Naloxone, then, could affect feeding by inhibiting central and/or peripheral effects of pituitary B-END, and/or by inhibiting the effects of centrally released B-END. At present there appears to be no way to determine whether the inhibiting effect of naloxone on food intake is central or

peripheral. An opiate antagonist that does not penetrate the blood-brain barrier would be most useful in resolving this question, but none seems to exist.

The preceding discussion assumes that the effects of naloxone are specific to opiate-activated feeding systems. This assumption is not unchallengeable. As indicated earlier, recent work (Frenk & Rogers, 1979) shows that one 10.0 mg/kg dose of naloxone is sufficient to condition a taste aversion in rats. It is possible, then, that higher (around 10.0 mg/kg and larger) doses of naloxone may suppress feeding simply by their noxious consequences, not by any effect specific to feeding systems.

The effects of lower doses of naloxone on food intake probably cannot be ascribed to aversive consequences of naloxone treatment. Frenk and Rogers (1979) found that 0.1 mg/kg naloxone was not sufficient to condition a taste aversion in rats. Further, LeBlanc and Cappell (1975) were not able to condition a taste aversion with naloxone at doses of 0.48, 1.44, or 4.32 mg/kg, even though six conditioning trials were used.

Even though the effects of low doses of naloxone on feeding behavior are probably not due to aversive states, it is still not clear that naloxone acts specifically on feeding mechanisms. Frenk and Rogers (1979) observed that 1.0 mg/kg naloxone inhibits water intake in rats deprived of water for

23 hours, as well as inhibiting food intake in rats deprived of food for the same amount of time. In fact, as little as 0.1 mg/kg naloxone suppressed water intake of 12-hour water deprived rats. Frenk and Rogers (1979) interpreted these findings as consistent with a hypothesis postulating opioid-mediated drive reduction systems (Belluzzi & Stein, 1977). According to this hypothesis, naloxone would inhibit feeding and drinking behaviors by blocking the normal drive reduction resulting from these consummatory behaviors. Drive reduction would be blocked because naloxone would inhibit the effect of opioids released by these behaviors. According to Frenk and Rogers (1979), these behaviors would be inhibited because they were no longer as rewarding as previously.

Frenk and Rogers' (1979) interpretation of the mechanism of naloxone's effect on food intake may provide an alternate interpretation for the data of Margules et al. (1978). Margules et al. (1978) reported that naloxone had a greater effect on the food intake of genetically obese rats than on the food intake of lean littermates. Margules et al. (1978) suggested that the enhanced sensitivity of genetically obese rats was related to the increased concentrations of pituitary B-END in these animals. It is possible, however, that the increased naloxone sensitivity of genetically obese rats might be due simply to obesity.

Food intakes of obese rats are more easily suppressed by dietary adulteration than are the food intakes of normals (Maller, 1964; Sclafani & Springer, 1976). Further, obese rats are less willing to work for food than normal weight rats (Sclafani & Springer, 1976). These observations could be interpreted as showing that obese rats have lower drive strengths for food than do normal rats under the same conditions. If naloxone inhibits food intake by making the consumption of food less rewarding, it might be reasonable to expect obese rats to be more sensitive to naloxone than normal rats. For obese rats, the consumption of food is already less strongly motivated than for normals, and therefore less rewarding. Further suppression of reward by naloxone might be evidenced in obese animals as a greater sensitivity to the effect of naloxone on feeding than is manifested by normal animals.

The idea that naloxone might differentially affect feeding in obese animals does not rule out the possibility that naloxone may also act directly on feeding-specific mechanisms involving opiate receptors. Central injections of B-END do, after all, stimulate feeding in normal weight animals, and this effect is blocked by naloxone (Grandison & Guidotti, 1977; Kenny et al., 1978). Further, the opiate antagonist methadone injected peripherally stimulates feeding (Holtzman, 1975). Methadone, B-END, and naloxone are all

known to interact largely with the same (opiate) receptors.

Effects of surgical treatment and diet on pituitary beta-endorphin-like immunoreactivity concentrations

The lack of a differential effect of naloxone among surgical groups may be more informative when considered in light of the assay data. Pituitary B-ENDLI concentrations were substantially lower in knife cut rats (68% of the mean of all other groups). If pituitary B-END is truly important in the regulation of feeding behavior, then the feeding of animals having elevated pituitary B-END concentrations might be more sensitive to naloxone antagonism. Further, the intakes of animals with decreased pituitary B-END concentrations might be less sensitive to naloxone antagonism. The report of Margules et al. (1978) that genetically obese mice and rats have both elevated pituitary B-END levels and increased sensivity to the feeding effects of naloxone lends support to this notion. However, in this experiment knife cut rats had much lower than normal concentrations of pituitary B-ENDLI, but were not less affected by naloxone than were other groups. The data from the knife cut rats, then, are contrary to the expectation of decreased naloxone sensitivity in rats with decreased pituitary B-END concentrations. It should be emphasized here that knife cut rats showed no trend which might differentiate their feeding responses to naloxone from those of the other

groups. The lack of a significant difference between knife cut and other groups does not appear to be due simply to excessive within group variance.

The significance of the substantially lowered (68%) B-ENDLI concentrations in knife cut rats is not immediately apparent. It is also difficult to resolve with the finding that VMH-lesioned rats had normal concentrations of pituitary B-ENDLI, since the feeding syndromes produced by these cuts and lesions are basically quite similar, although not identical (e.g. Peters, Wellman, & Gunion, 1979). This unexpected dissimilarity between knife cut and VMH syndromes may be related to another unexpected dissimilarity recently observed by Bray, Sclafani, and Novin (Note 1). Bray et al. (Note 1) found that knife cut rats held at normal body weight were not hyperinsulinemic. This is in marked contrast to the typical finding that VMH rats are hyperinsulinemic under these conditions (Friedman & Stricker, 1976). A possible link between the lack of hyperinsulinemia (Bray et al., Note 1) and the lowered pituitary concentration of B-ENDLI in knife cut rats may be found in the work of Ipp et al. (1978). Ipp et al. (1978) reported that B-END causes release of insulin from pancreatic islets in vitro, suggesting that B-END is a regulator of insulin secretion. It may be, then, that the lower than normal pituitary B-ENDLI concentration of knife cut rats adversely affects insulin synthesis and/or

release in these animals. It would be interesting to know if B-END has a trophic effect on the islets of Langerhans.

In evaluating the role of pituitary B-END in hyperphagia and obesity development, it may be worthwhile to further examine the data of Margules et al. (1978) in conjunction with the data reported here. Genetically obese rats (Margules et al., 1978), VMH-lesioned rats, DLT-lesioned rats, and knife cut rats all eat more than do normal animals, and will all weigh more than normals if allowed to eat freely. Pituitary B-END concentration is normal in VMH and DLT rats, substantially elevated in genetically obese rats (75%), and substantially depressed in knife cut rats (68%). These comparisons strongly suggest that there is no necessary link between pituitary B-END concentrations and feeding activity. It may be that genetic obesity is indeed due to elevated pituitary B-END concentrations, as suggested by the data of Margules et al. (1978); however, it certainly appears that not all overeating/obesity syndromes are associated with elevated pituitary B-END concentrations.

Regression analysis showed that diet had a statistically significant effect on pituitary B-ENDLI concentration, with animals restricted to the pellet diet showing slightly (11%) lower concentrations of pituitary B-ENDLI than animals allowed to feed freely on the wet mash diet. This small difference is most likely an effect of diet per se rather

than body weight or feeding regimen. FF rats maintained on pellets (which always had free access to food) had concentrations of pituitary B-ENDLI 14% lower than those of FF rats switched to the wet mash diet. Why the wet mash diet should alter pituitary B-ENDLI concentration is not clear.

Food intake, body weight, and the wet mash diet

The changes in body weight and food intake during maintenance on the wet mash diet were generally as expected. Both VMH and KC groups increased their food intakes substantially (204% and 210%, respectively, of NWRA intake). By day 36 both these groups weighed 168% of the weight of group NWRA. Groups DLT and OVX also ate more than group NWRA (132% and 121%, respectively, of NWRA intake), and weighed more than NWRA on day 36 (111% and 114%, respectively, of NWRA). Also as expected, neither group FF or DEP ate more than NWRA over the 36 days, and neither of these groups weighed significantly more or less than NWRA on day 36.

The feeding and weight changes of group 5,7-DHT were not as expected. Rats treated with 5,7-DHT have previously been shown to overeat and gain weight when allowed free access to either a pellet (Saller & Stricker, 1976) or high fat (Luttmers, 1978) diet. In this experiment 5,7-DHT rats did not overeat or gain weight to greater than control (NWRA) levels when allowed free access to a wet mash diet. This was surprising, since these rats ate more than NWRA rats during

restricted access testing using pellets. Since 5,7-DHT rats will overeat under ad libitum conditions, it is most likely that the wet mash diet was the proximate cause of the failure to overeat. Why this should be is not clear. In general, wet mash diets induce larger weight gains than pellet diets (e.g. Peters, Wellman, & Gunion, 1979), and the wet mash diet was used here for that reason. It may be that 5,7-DHT rats have trouble eating wet mash. During this experiment 5,7-DHT rats left large amounts of spillage beneath their cages during restricted access testing with pellets. Luttmers (Note 2) also noted greater than normal spillage under 5,7-DHT cages when a high fat diet was used. The spillage here was composed of larger "shreds" of food pellets, rather than the crumb- or dust-like spillage of all other groups in this experiment. This shredding of food may indicate physical difficulty in biting, chewing, or some other aspect of food handling. Alternatively, the large water content of the wet mash diet may pose difficulties for 5,7-DHT rats. Central mechanisms are most certainly involved in the regulation of body water and electrolyte balance. Treatment with 5,7-DHT may damage one or some of these mechanisms, making it simply impossible for 5,7-DHT treated rats to accommodate very large water intakes.

One interesting finding of this experiment was that rats given intraventricular injection of 5,7-DHT did overeat

during the restricted access testing periods, which occurred during the dark portion of the daily light/dark cycle. Rats given such treatment and allowed continuous access to food have been shown to overeat only during the light portion of the daily cycle, and not during the dark (Saller & Stricker, 1976). The results of the present experiment suggest that this circadian rhythmicity may be altered by restricted access feeding. These results are also interesting in light of some failures to replicate the initial report of Saller and Stricker (1976). Both Coscina and Hoebel (Coscina, 1978; Hoebel, Zemlan, Trulson, MacKenzie, DuCret, & Norelli, 1978) reported that injections of 5,7-DHT did not result in increased feeding or weight gain. Luttmers (1978) did replicate the findings of Saller and Stricker (1976), and may be the only replication to date.

Summary

None of the experimental hyperphagia/obesity syndromes examined here was more or less sensitive than normal to the food intake suppressing effect of the opiate antagonist naloxone. Rats with hypothalamic parasagittal knife cuts had substantially lowered concentrations of pituitary B-ENDLI, while other hyperphagia/obesity syndromes examined did not show altered B-ENDLI levels. Obesity did not significantly alter pituitary B-ENDLI concentrations. In conjunction with data from genetically obese rats, these data indicate that

elevated, or even merely altered, concentrations of pituitary B-END are not necessarily related to hyperphagia and obesity.

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Appendix: Literature Review

Four years ago a new class of brain penta-peptides having opioid properties, the enkephalins, was discovered (Hughes, Smith, Kosterlitz, Fothergill, Morgan, & Morris, 1975). With this discovery it was observed that one of these penta-peptides was found in beta-lipotropin (B-LPH), a polypeptide previously isolated from camel pituitary and sequenced (Li & Chung, 1976). It was soon found that another closely related class of polypeptide also existed (Ling, Burgus, & Guillemin, 1976). The members of this class, the endorphins, were between 16 and 31 amino acids long, were also contained within the sequence of B-LPH, and themselves all contained one of the enkephalins (met-enkephalin) as their five N-terminal amino acids.

Although these substances were initially investigated with regard to their opioid-like analgesic properties (e.g. Cox, Goldstein, & Li, 1976; Graf, Szekely, Ronai, Dunai-Kovacs, & Bajusz, 1976), other effects have been examined. The enkephalins and endorphins have now been investigated with regard to mental illness (Vereby, Volavka, & Clouet, 1978: Watson, Akil, Berger, & Barchas, 1979), sexual behavior (Gessa, Paglietti, & Quarantotti, 1979), open field activity (Veith, Sandman, Walker, Coy, & Kastin, 1978), stress responses (Guillemin, Vargo, Rossier, Minick, Ling, Rivier, Vale, & Bloom, 1977), the regulation of feeding

(Kenny, McKay, Woods, & Williams, 1978; Margules, Moisset, Lewis, Shibuya & Pert, 1978), and digestive activities (Hughes et al., 1975; Jacques, 1977; Konturek, Pawlik, Walus, Coy, & Schally, 1978; Konturek, Tasler, Cieszkowski, Jaworek, Coy, & Schally, 1978). The feeding and digestive effects are of interest here. Before these effects can be described, however, it is necessary to briefly differentiate among the opioid peptides, and to describe their general distribution in the body.

Biosynthesis and distribution

Biosynthesis. The interrelationships among the enkephalins, endorphins, and their precursors are complex. The essential relationships will be outlined here.

The parent of at least some of these substances is a 31,000 dalton glycoprotein that has been termed "proopiocortin" (Mains, Eipper, & Ling, 1977; Rubinstein, Stein, & Udenfriend, 1978). This term is quite appropriate, since this molecule is the parent to adrenocorticotropic hormone (ACTH) as well as some of the opioid peptides. Proopiocortin is apparently fragmented to form ACTH and B-LPH. It was initially thought that B-LPH, in turn, was fragmented to directly form the endorphins: alpha-endorphin (B-LPH 61-76), beta-endorphin (B-LPH 61-91; B-END), and gamma-endorphin (B-LPH 61-77). Although B-END does appear to be a true product of B-LPH degradation (Crine, Benjannet,

Seidah, Lis, & Chretien, 1977; Graf, Cseh, Barat, Ronai, Szekely, Kenessey, & Bajusz, 1977), data now indicate that alph-endorphin may be an extraction artifact (Rossier, Bayon, Vargo, Ling, Guillemin, & Bloom, 1977). It also appears that gamma-endorphin is a degradation product of B-END (Graf et al., 1977), rather than a direct product of B-LPH.

The sequence of met-enkephalin, one of the two known naturally occurring opioid penta-peptides, is found as the N-terminal five amino acid sequence of B-END (B-LPH 61-65; Tyr-Gly-Gly-Phe-Met; Hughes et al., 1975). There is very suggestive evidence, however, that met-enkephalin is not normally formed intracellularly by B-END degradation (Bloom, Battenberg, Rossier, Ling, & Guillemin, 1978; DiGuilo, Lutold, Fratta, Yang, & Costa, 1978; Polak, Bloom, Sullivan, Facer, & Pearse, 1977; Schulz, Wuster, Simantov, Snyder, & Herz, 1977; Watson, Akil, Richard, & Barchas, 1978), although it may be formed in that manner extracellularly (Austen, Smyth, & Snell, 1977). The other enkephalin, leu-enkephalin (Tyr-Gly-Gly-Phe-Leu; Hughes et al., 1975) is not found in the sequence of any known portions or products of proopiocortin. It is thought that both enkephalins may be synthesized by some other route (Watson, Barchas, & Li, 1977; Watson et al., 1978).

Distribution. B-END has been located in both brain and pituitary (Bloom, Rossier, Battenberg, Bayon, French,

Henriksen, Siggins, Segal, Browne, Ling, & Guillemin, 1978). In brain, B-END is found in greatest amounts in the hypothalamus, with lesser concentrations in the septum, midbrain, and medulla-pons. Hypothalamic neurons containing B-END have been located by immunohistofluorescence along the third ventricle within the periventricular and arcuate nuclei, and along the base of the brain moving laterally from the arcuate nucleus, and terminating ventral to the lateral hypothalamic area. Some B-END neurons are found along the thalamic third ventricle, and within the thalamic paraventricular nucleus (Bloom, Rossier, Battenberg, Bayon, French, Henriksen, Siggins, Segal, Browne, Ling, & Guillemin, 1978).

B-LPH, the apparent precursor to B-END, has been located in the same regions as B-END; additionally, it has been found that the locus coeruleus is heavily invested by B-LPH neurons, as is the central gray around the cerebral aqueduct. B-LPH has also been located in the zona incerta, ansa lenticularis, stria terminalis, and medial amygdala. Moderate investment of B-LPH neurons is found in the substantia nigra zona compacta (Watson et al., 1977).

Pituitary B-END is found in highest concentrations in the pars intermedia (1500 ng/g tissue, rat), with pars distalis showing some B-END (269 ng/g tissue, rat). No B-END has been found in pars nervosa (Bloom, Rossier, Battenberg,

Bayon, French, Henriksen, Siggins, Segal, Browne, Ling, & Guillemin, 1978). This quantitative work correlates well with immunohistofluorescence work showing that all pars intermedia cells contain B-END/B-LPH, while scattered B-END/B-LPH cells are seen in the pars distalis (Pelletier, Leclerc, Labrie, Cote, Chretien, & Lis, 1977; Watson et al., 1978). It has also been shown that B-END, B-LPH, and gamma-endorphin are synthesized in bovine pars intermedia in vitro (Crine et al., 1977).

Neither of the enkephalins have been found in pituitary tissue (Bloom, Rossier, Battenberg, Bayon, French, Henriksen, Siggins, Segal, Browne, Ling, & Guillemin, 1978; Watson et al., 1978). The enkephalins do occur in brain, but show a distribution distinctly different from B-END and B-LPH (Bloom, Rossier, Battenberg, Bayon, French, Henriksen, Siggins, Segal, Browne, Ling, & Guillemin, 1978; Johansson, Hokfelt, Elde, Schultzberg, & Terenius, 1978; see also Watson et al., 1978). The enkephalins have also been found in gut, primarily in the antrum and the upper small intestine. In the antrum enkephalin is found in gastrin-secreting cells, but not in cells secreting other peptides (e.g. somatostatin) (Polak et al., 1977). In the small intestine enkephalin is found both within the myenteric plexus and within the circular muscle (Johansson et al., 1978); it has been shown that enkephalin is released in vitro from electrically

stimulated myenteric plexus-longitudinal muscle preparations (Hughes, Kosterlitz, & Sosa, 1978; Schulz et al., 1977). Enkephalin has also been found in the pancreas, gallbladder, and colon (Polak et al., 1977). It should be noted that the physical location of the enkephalins in the gut corresponds well to their apparent physiological effects (see below). B-END and B-LPH have not been found in the gut, except in a few cases of ectopic ACTH secreting tumors (Hirata, Matsukura, Imura, Nakamura, & Tanaka, 1976; see also Guillemin et al., 1977).

Role of opioid peptides in the physiology and behavior of food intake and digestion

Enkephalins. The enkephalins have interesting effects in the gut. Met-enkephalin increases the rate of gastric blood flow when tested alone, and enhances gastric acid secretion induced by histamine (Konturek, Pawlik, Walus, Coy, & Schally, 1978). As stated earlier, enkephalin has been found in antral gastrin secreting cells (Polak et al., 1977); such a close relationship with gastrin, both physically and physiologically, suggests the two substances may act synergistically in the control of gastric acid secretion.

The location of enkephalin in the intestine (Johansson et al., 1978) may be related to its effect in inhibiting contraction of electrically (Hughes et al., 1975) or chemically (Jacques, 1977) stimulated myenteric

plexus-longitudinal muscle preparations. Likewise, location of enkephalin in the pancreas may be related to the effect of exogenous enkephalin in decreasing the bicarbonate and exocrine protein secretion of that organ (Konturek, Tasler, Cieszkowski, Jaworek, Coy, & Schally, 1978).

Aside from these effects on gut physiology, the enkephalins have not been shown to have an effect, or potential effect, on food intake.

Endorphins. Indirect evidence for the role of a pituitary factor in the regulation of feeding, possibly B-END, comes from several lines of research.

Beloff-Chain and colleagues have shown that a factor released from mouse pituitary in vitro causes release of insulin from isolated pancreatic islets (Beloff-Chain, Edwardson, & Hawthorn, 1975; Beloff-Chain, Edwardson, & Hawthorn, 1977; Beloff-Chain & Hawthorn, 1976; Beloff-Chain, Hawthorn, & Green, 1975). Perifusate from pituitaries of genetically obese mice causes a greater release of insulin than does perifusate of normal mouse pituitaries when normal islets are used (Beloff-Chain, Hawthorn, & Green, 1975). This pituitary factor apparently affects a quickly mobilized insulin pool, and pancreatic islets from genetically obese mice appear refractory to this factor (Beloff-Chain & Hawthorn, 1976).

It is also known that hypophysectomized rats have a decreased insulin response to glucose challenge (Malaisse, Malaisse-Lagae, King, & Wright, 1968; Randle & Young, 1956). Such a decreased insulin response suggests that the presence of some pituitary factor(s) is (are) necessary for the normal maintenance of insulin synthesis and/or release. Whether this effect is entirely due to any of the previously identified pituitary hormones is not clear (cf. Malaisse et al., 1968, and Randle & Young, 1956). Beloff-Chain (Beloff-Chain et al., 1977) has suggested that this pituitary factor is corticotropin-like intermediate lobe peptide (CLIP; ACTH 18-39), since (1) CLIP and ACTH 17-39 cause insulin release in vitro (ACTH does not); (2) the unknown pituitary factor Beloff-Chain found to cause insulin release was found only in pars intermedia perifusate; and (3) CLIP is found in the pars intermedia (Lowry, Silman & Hope, 1977). While CLIP would thus appear a likely candidate, it must be noted that CLIP may not exist under normal physiological conditions, except during pregnancy (Lowry et al., 1977). As noted in a previous section, B-END is also found in the pars intermedia (Bloom, Rossier, Battenberg, Bayon, French, Henriksen, Siggins, Segal, Browne, Ling, & Guillemin, 1978), and in fact exists there in the highest concentration of any tissue yet examined quantitatively. B-END, then, might be a candidate for the insulin releasing

factor of Beloff-Chain. Recently Ipp, Dobbs, and Unger (1978) reported data supporting this possibility. They reported that B-END can cause release of insulin from pancreatic islets in vitro. This finding suggests that elevated pituitary B-END and a resultant hyperinsulinemia may play an important role in genetic obesity.

Direct evidence linking pituitary B-END to the regulation of food intake comes from a recent study of genetically obese mice and rats (Margules et al., 1978). Margules found that genetically obese rats and mice have pituitary B-END levels 75% and 80%, respectively, above those of lean littermates. Further, it was found that the exaggerated food intake of these animals was antagonized to a greater degree than that of lean littermates by injection of the opiate antagonist naloxone. In rats every dose tested (0.25 to 1.0 mg/kg ip) produced a greater percent reduction of food intake in obese animals than their lean littermates. Similar results were obtained in the genetically obese mice.

Data suggesting that some substance that interacts with opiate receptors is involved in the normal regulation of food intake has been obtained by Holtzman (1974, 1975) and Margules et al. (1978). Holtzman (1974) showed that naloxone produced a dose-dependent decrease in intake of regular laboratory food pellets in normal rats, finding that a dose of 10 mg/kg practically abolished food intake (7% of saline

control). Holtzman (1975) showed that naloxone, naltrexone, and nalorphine, all opiate antagonists, reduced consumption of sweetened Enfamil in a dose-dependent manner. Further, the opiate agonist methadone caused an increase in intake when given at a low dose (0.1 mg/kg). These apparently clear-cut results are tempered, however, by the finding that neither morphine nor cyclazocine, two opiate agonists, caused an increase in intake at any dose tested. Margules et al. (1978) found naloxone decreased food intake in lean rats and mice as well as in genetically obese ones, but that lean rodents required higher doses.

Direct central manipulations have also yielded data indicating a possible role for B-END in the regulation of feeding. Kenny et al. (1978) found that injection of 200 ng B-END into the lateral ventricle of the rat increased comsumption of sweetened milk. This effect was not seen with cholecystokinin, substance P, or neurotensin. Peripheral injection of the same dose was without effect. Grandison and Guidotti (1977) found that intrahypothalamic injection of 1.46 nm of B-END caused increased intake of laboratory chow. The feeding effects of B-END were blocked by subsequent intrahypothalamic injection of naloxone. Grandison and Guidotti (1977) also reported data suggesting that GABA-receptive neurons serve as mediators for feeding due to intrahypothalamic application of either B-END or

norepinephrine.

Summary

1. B-END is known to directly stimulate feeding when injected centrally, and neurons containing B-END exist in or near these areas.
2. A mechanism exists for peripheral (pituitary) B-END to cause feeding by direct stimulation of insulin release.
3. Genetically obese rats and mice have increased levels of pituitary B-END.
4. Naloxone, which blocks B-END effects by competitive inhibition:
    a. suppresses feeding in normal animals;
    b. suppresses intake in animals feeding due to B-END injection;
    c. is more effective in suppressing feeding of genetically obese rats and mice than in their lean littermates.
5. Enkephalins may well play a role or roles in the local regulation of gut activities, but at present do not otherwise seem to be involved in feeding or related activities.

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